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Temporal map formation in appetitive second-order conditioning in rats

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Running Head: Temporal maps

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Abstract

Three experiments examined whether second-order conditioning resulted in the formation of a fully-featured temporal map, as proposed by the temporal coding hypothesis. Experiments 1 and 2 examined second-order conditioning with different first- and second-order relationships. Measures of the strength of second-order conditioning were mostly consistent with the temporal coding hypothesis; second-order conditioning was best with arrangements in which CS2 occurred prior to the time that the US normally occurred during CS1-US presentations. However, there was no evidence of anticipatory timing during CS2 during second-order conditioning. A third experiment directly examined whether a fully-featured temporal map was formed during second-order conditioning by examining the acquisition of anticipatory timing in subsequent reinforced second-order trials. The results of Experiment 3 suggested that the effects obtained in Experiments 1 and 2 were due to learning of the temporal order and coincidence of events that resulted in the formation of an ordinal temporal map, but that precise durations were not encoded.

Keywords: second-order conditioning; appetitive conditioning; timing; temporal map; rats

During a standard Pavlovian conditioning task, a neutral event (the conditioned stimulus, CS) is paired with an outcome (the unconditioned stimulus, US). After several such pairings, the CS comes to elicit anticipatory conditioned responses (CRs) indicating that the subject has learned to associate the two events. Second-order conditioning (SOC) involves presentations of a conditioned stimulus followed by an unconditioned stimulus such as food or shock in a standard conditioning arrangement. In the key second phase, the original CS (CS1) is now paired with a novel CS (CS2) in the absence of the US. Second-order conditioning is apparent if responding is observed to CS2, even though this stimulus was never directly presented with the US [1].

Second-order conditioning is a specific instance of a set of conditioning paradigms that are thought to necessitate integration of information across phases [see, for example, 2]. In the case of SOC, the activation of a previously learned CS1→US relationship during the CS2→CS1 pairing phase may allow for determination of the CS2→US relationship. While there is evidence that temporal integration may occur [e.g., 3, 4], whether precise temporal information is transferred between phases in SOC is unknown. Answering this more challenging question requires comparison of different temporal arrangements of CS1 and CS2, an issue that has been largely overlooked in the literature. Rats do learn to time the duration of the CS, such that the distribution of the CR peaks at about US delivery [e.g., 5], suggesting that specific temporal information is available for transfer. Any effect of order and/or timing of events may support the notion that temporal information was transferred, and critically the nature of these effects could indicate the degree of specificity of information that is integrated across phases. A common approach to the study of order and timing effects has been to alter the CS1-CS2 relationship during the second-order phase. Various manipulations with simultaneous CS1/CS2 [e.g., 6, 7], backward CS1→CS2 [e.g., 8, 9], and forward CS2→CS1 [e.g., 6, 7, 9, 10, 11] presentations have shown at least some evidence for SOC, with successful conditioning most often observed with forward presentations.

The most systematic approach to this question has been that of Miller and colleagues [e.g., 3, 4, 6, 12], which led to the development of the *temporal coding hypothesis* [13, 14]. The temporal coding hypothesis proposes that second-order conditioning is promoted by the formation of a temporal map that encodes the CS1→US interval and CS2→CS1 interval. Through the process of temporal integration in memory, the CS2→US interval is determined. If the CS2→US interval is an arrangement that would normally result in the expression of CRs in first-order conditioning, then CRs will be observed in SOC. For example, Cole, Barnet, and Miller [4] initially trained rats with a delay conditioning (5-s CS1 → shock US) or trace conditioning (5-s CS1 → 5-s trace → shock US) arrangement. SOC involved CS1→CS2 presentations in the absence of shock. Critically, the trace conditioning group displayed better SOC even though this group exhibited poorer first-order conditioning. Cole et al. [4] argued that the trace group would have formed a CS1-CS2-US map in which CS2 was in a forward relationship with the US. On the other hand, the delay group would have formed a CS1-US-CS2 map in which CS2 was in a backward arrangement with the US, which would not result in very robust conditioning. One key factor in this experiment is that there was a built-in control for generalization (although there may have been differences in contextual conditioning): the trace group should have exhibited weaker generalization because of their weaker first-order conditioning, so generalization alone cannot account for the pattern of results.

An important feature of the temporal coding hypothesis is the notion that SOC is promoted by temporal integration. However, it is not clear whether temporal integration necessitates precise timing information. Kirkpatrick and Balsam [15] described two alternative conceptual frameworks within which temporal maps may reside. One possibility is that temporal maps rely on detection of coincidence and order of events, but that the timing of events would rely on a separate delay processing module. Alternatively, temporal maps may contain fully-integrated, feature-rich information encoding of coincidence, order, and timing information. The present series of experiments sought to determine whether second-order conditioning involves the generation of a fully-featured temporal map. Such a map should reveal itself not only in the effectiveness of SOC as a function of the order in which the stimuli are presented, but also in the temporal gradients (timing) of responding during CS2. If rich temporal information is acquired in SOC, one would expect to see a temporal gradient during CS2 that peaks near the expected time of US delivery. To investigate whether a fully featured temporal map is indeed acquired, we delivered SOC under different arrangements and examined both the strength (conditioning) and timing (distribution) of CRs.

Experiment 1

Experiment 1 examined SOC in an appetitive conditioning paradigm with rats. All the rats received first-order conditioning (FOC) with a 10-s CS1 followed by food. SOC involved nonreinforced presentations of a 10-s CS2 together with the 10-s CS1. Four different arrangements were employed: forward CS2→CS1, backward CS1→CS2, simultaneous CS1+CS2, and unpaired CS1~CS2. These temporal arrangements were chosen because they have been commonly employed arrangements in prior investigations because they provide controls for non-associative effects such as habituation, but only rarely have they been directly compared.

Based on the temporal coding hypothesis, we predict that only the forward and simultaneous arrangements should result in SOC, because these arrangements result in predictive information (e.g., CS→US) that is effective for FOC [e.g., 3]. The unpaired group provides a control for generalization against which to assess the other three groups. Experiment 1 also examined timing during CS2-only peak trials to determine whether the rats express learning of the CS2 onset-***US***[1] interval. This interval is 20 s in the forward arrangement and 10 s in the simultaneous arrangement. Thus, one would expect to observe peaks in responding during CS2 around these times.

Method

Animals. The subjects were 24 experimentally-naïve male Sprague-Dawley rats (Harlan UK). On arrival, the rats weighed 120-140 g; they were housed in pairs based on initial weight. The rats were given ad libitum food access for one week, after which they each received 15 g of food per day. Water was freely available in the home cages and experimental chambers. All rats received handling each day beginning three days after arrival to the colony room.

Apparatus. The experimental procedures were conducted in twelve identical chambers (25 x 30 x 30 cm), each of which was situated within a ventilated, noise-attenuating box (74 x 38 x 60 cm). The chambers were in two rooms with six chambers per room. Each chamber was equipped with a speaker for delivering auditory stimuli, a houselight, a food cup, and a water bottle. The speaker was located on the right side of the back wall of the chamber, on the opposite wall from the food cup. The houselight was situated on the top-center of the wall above the food cup. A magazine pellet dispenser (Model ENV-203) delivered 45-mg Noyes (Improved Formula A) pellets into the food cup. Each head entry into the food cup was transduced by an LED-photocell. The water bottle was mounted outside the chamber; water was available through a tube that protruded through a hole in the lower-center of the back wall of the chamber. Med-PC for Windows [16], running on two Pentium III 800-mHz computers (one for each set of six chambers), controlled experimental events, and recorded the time at which events occurred with 2-ms resolution.

Procedure. Each animal was randomly assigned to one of four groups (*n* = 6) – Simultaneous, Backward, Forward, or Unpaired. The timing and order of CS and US presentations during first- and second-order conditioning is displayed in Figure 1. The arrows below each procedural diagram indicate the intervals that could be learned directly in FOC (solid lines) and indirectly through temporal integration in SOC (dashed lines).

First-order conditioning (Sessions 1-8). All rats received 8 sessions of first-order conditioning (FOC) that comprised 32 trials with a fixed duration 10-s houselight stimulus, immediately followed by the delivery of a 45-mg Noyes pellet (see Figure 1). The time from stimulus termination (or food delivery) to the next stimulus onset, the intertrial interval (ITI), was an exponentially distributed random interval with a mean of 200 s and a minimum of 100 s.

Second-order conditioning (Sessions 9-18). The procedure for SOC was adapted from Hatfield et al. [17]. SOC followed the last session of FOC and consisted of 10 sessions of 16 trials, each with the same ITI as in FOC. A 10-s, 70-db white noise that served as CS2 was introduced during this phase (see Figure 1). In Group Simultaneous, the light and noise were presented simultaneously. In Group

Backward, the onset of the noise stimulus followed immediately after the houselight switched off (CS1→CS2). In Group Forward, the opposite stimulus arrangement was presented. Group Unpaired received unpaired presentations of the noise and light stimuli; the two stimuli were separated by an exponentially-distributed random interval with a mean of 100 s and a minimum of 50 s, with a pseudo-random determination of which stimulus would occur during the specified time, with the constraint that the total number of CS1 and CS2 occurrences had to be equal. Testing of SOC was achieved by recording both the magnitude and pattern of responding during 30-s peak trials, which consisted of non-reinforced noise only presentations.

The first three sessions of SOC (9-11) began with two reinforced FOC trials to maintain responding. During the remainder of the session, there were three different trial types that were randomly intermixed: two further FOC trials, ten non-reinforced SOC trials, and two non-reinforced 30-s CS2-only peak trials. Following these three sessions, two further sessions of SOC (12-13) were delivered in which the two initial FOC trials were omitted and the number of peak trials was increased to four; the number of SOC trials was maintained at 10 and all trial types were randomly intermixed. This manipulation was conducted to increase the number of peak trials to improve the stability in the timing functions. For the remaining five sessions of SOC (14-18), two extra FOC trials were delivered randomly in the session returning the total number of FOC trials to four and two SOC trials were removed; this change was made because response rates had drifted downwards.

Reinforcement of the second-order compound (Sessions 19-38). The analysis of SOC in Sessions 9-18 indicated a lack of timed responding in all conditions. Accordingly, sessions 19-28 delivered two randomly-selected SOC trials that were reinforced (SOC+ trials), to determine whether occasional food delivery might improve timing during the peak trials. Food delivery occurred at the time of CS1 termination in the SOC+ trials (see dashed open triangles in Figure 1). The procedure was then modified during Sessions 29-38 so that all SOC trials ended in reinforcement. Throughout this phase, all other aspects of the procedure were delivered as during Sessions 14-18 of second-order conditioning.

Data Analysis

The time of occurrence of each stimulus onset, stimulus termination, food delivery, and head entry response was recorded with a time stamp, with a resolution of 2 ms. Summary data were extracted from the raw data using MATLAB (The Mathworks, Natick, MA) to assess conditioning and timing of the two stimuli. Statistical analyses were conducted in SPSS. All sessions within a phase were included and analyses collapsed across sessions within a phase. Test statistics are reported for significant effects only, with an alpha level of .05.

Conditioning was measured by determining the mean response rate during CS1 on FOC conditioning trials, and during peak trials for CS2 in SOC. Analyses of temporal control of behavior were conducted by determining the rate of responding in successive 1-s time bins. These functions were then normalized by dividing the response rate in each time bin by the maximum of the response rate function. Normalized rates have traditionally been used to analyze peak response functions when there are differences in response rates among individuals or across groups as they allow for analysis of the shape of the response functions independent from the response rates [e.g., 18]. Accordingly, normalized rates were calculated so that each rat would contribute equally to the group timing functions, regardless of its overall response rate. To examine temporal control during SOC, we calculated linear response slopes for each rat as the response slope varies systemically with interval duration and is therefore an indicator of the interval that is being timed [5]. The number of responses occurring in each 1-s time bin were fit with a linear function and a slope was calculated for the first 10 s and first 20 s of CS2. We selected these two durations to estimate the temporal control by the CS2-US durations in line with the expectations of the temporal coding hypothesis (e.g., 10 s for Group Simultaneous and 20 s for Group Forward). Group comparisons of the slopes were conducted using an ANOVA. In addition, one-sample t-tests were conducted to compare the slopes to zero. A positive slope indicated evidence of timing of the CS2-***US*** interval. Conversely, a negative or zero slope indicated a lack of temporal control.

Results and Discussion

First-order conditioning. First-order conditioning resulted in the acquisition of conditioned responding during the light CS1 that was temporally dependent. These data are not shown, but the timing functions are comparable to those that were taken from FOC trials during SOC (see below). The mean rate of responding to the 10-s CS did not differ significantly among the four groups (p = .225, η^2 = .19) during FOC (lowest mean: 25.8 ± 2.6 responses/min.; highest mean: 33.0 ± 4.4 responses/min.). One would not expect any differences in responding during FOC because all four groups received the same procedure.

Second-order conditioning.

Conditioning to CS2. The mean rate of responding during the first 10 s of CS2 peak trials[2] in SOC is shown in Figure 2 for each group. As seen in the figure, the rate of responding in Group Forward was higher than the other three groups. This was verified by an ANOVA conducted on the group mean response rates, collapsed across the 10 sessions, which revealed a significant main effect of group, $F(3,20)$ = 11.21, p < .001, η^2 = .63. Post-hoc tests (Tukey HSD), confirmed that Group

Forward differed from the other three groups ($p < .01$) and that Groups Simultaneous, Backward, and Unpaired did not differ from one another.

The observation of SOC in Group Forward, as opposed to Groups Backward and Unpaired is consistent with the temporal coding hypothesis because Group Forward experienced a forward CS2-***US*** relationship (a 20-s CS onset-***US*** interval). This finding therefore supports the notion that a temporal map was formed across first- and second-order conditioning phases. However, the lack of acquisition in Group Simultaneous is somewhat puzzling because the CS2 onset-***US*** interval was 10 s and should have resulted in robust conditioning. It is possible that the failure in Group Simultaneous was due to the rapid development of conditioned inhibition, which appears to occur more readily in simultaneous compounds than in serial compounds [6]. Alternatively, CS1 may have impaired CS2 acquisition during SOC due to their simultaneous pairing because CS2 provided redundant temporal information regarding the prediction of the US. Finally, poor SOC in the simultaneous compound may have been due to a generalization decrement on CS2 only trials because CS2 did not normally occur on its own. Overlapping compounds will be examined further in Experiment 2 to test these latter two possibilities.

Timing of CS1 and CS2 duration. Figure 3 displays the normalized response rates (percentage of maximum rate, see *Data Analysis*) during light FOC trials (dashed lines) and nonreinforced noise-only SOC peak trials (solid lines) collapsed across Sessions 9-18 of SOC. The response rate during the FOC stimulus is similar for all four groups, with an increase in responding that reached a maximum near the end of the 10-s duration. Thus, it appeared that the rats timed the 10-s light duration throughout SOC.

On SOC peak trials, the expected time of a peak in responding is marked by an arrow for groups that had a forward CS2→***US*** relationship. Visual inspection of the response distributions indicates that the rats in groups Simultaneous, Backward and Unpaired responded in a similar fashion, with some initially higher responding during the first 10 s of the peak trial, followed by a decrease in responding. Group Forward on the other hand, showed an increase in responding over the initial few seconds of the peak trial, after which there was a gradual decline. Their peak was, however much earlier than the expected peak at 20 s. Thus, it appears that although Group Forward demonstrated evidence of SOC, they did not accurately time the CS2 onset-***US*** interval. An examination of the figure reveals that these animals were not simply timing the CS1 onset-US duration on noise peak trials because the form of the peak function is discernibly different from the form of the response function on light FOC trials (dashed lines). The relatively poor timing in Group Forward may have been due to a performance deficit, caused by the absence of reinforcement. Kirkpatrick and Balsam [15] argued that CRs driven by temporal maps may be acquired but behaviorally silent, with expression only occurring when conditions later become advantageous for expression of the temporal map knowledge, such as under conditions of reinforcement. Therefore, timing was assessed when the second-order compound trials were reinforced.

Reinforcement of the second-order compound. The introduction of occasional reinforced SOC trials (a random 2 trials per session) occurred first to see whether the presence of reinforcement on these trials would aid the expression of timing. However, there was no effect of this manipulation on the shape of the response rate function (data not shown).

Therefore, 10 sessions were administered with all second-order compound trials ending in reinforcement. The results of this manipulation are shown in Figure 4, with arrows indicating the expected time of the peak in responding on the nonreinforced CS2 peak trials. The response on peak trials was similar to the SOC phase in Groups Simultaneous, Backward, and Unpaired, but Group Forward displayed a well-defined peak. Reinforcement sharpened the peak and resulted in a rightward shift, but the peak was still before the expected time of reinforcement at 20 s. A comparison of the response slopes at 10 and 20 s revealed a significant Group effect, $F(3,20) = 7.87$, $p = .001$, $\eta^2 = .54$, and $F(3,20) = 7.60$, $p = .001$, $\eta^2 = .53$, respectively. Tukey post-hoc tests showed that Group Forward had a significantly more positive slope than Groups Backward and Simultaneous at both 10 and 20 s ($p < .01$). One sample t-tests indicated that Group Forward had a significant positive slope at both 10 s, $t(5) = 5.57$, $p = .003$, and 20 s, $t(5) = 3.99$, $p = .010$. The slopes for the other three groups did not differ from zero.

The slope analysis indicated that only Group Forward acquired temporal control over their responding on peak trials. To further assess the accuracy of timing in this group, the median response time [19] on peak trials was computed. The median response time is the time when the middle response occurs within a trial, which provides an index of the center (i.e., middle time) of the response function. The median response time for Group Forward was 15.3 $\pm$ .8 s during the reinforced compound training phase. One-sample t-tests indicated that the median response time was significantly later than 10 s, $t(5) = 6.64$, $p = .001$, and was significantly earlier than 20 s, $t(5) = -5.81$, $p = .002$.

The slope during the first 10 s of CS2 was compared against the slope during CS1 to assess early CS2 timing in relation to CS1. The ANOVA revealed a significant effect of Stimulus, $F(1,20) = 146.68$, $p < .001$, $\eta_p^2 = .88$, which was due to steeper slopes to CS1. There also was a main effect of Group, $F(3,20) = 4.53$, $p = .014$, $\eta_p^2 = .40$, which due to Group Forward having steeper slopes overall compared to the other three groups. Finally, there was a Group × Stimulus interaction, $F(3,20) = 3.30$, $p = .042$, $\eta_p^2 = .33$. Additional pairwise comparisons were conducted to compare the slopes to CS1 versus CS2 in the four groups. All groups showed significantly steeper slopes to CS1 compared to CS2, $ts(5) \geq 4.56$, $ps \leq .006$. However, the magnitude of the difference in slopes was smaller for Group Forward compared to the other three groups, which is the likely source of the interaction. One would expect that Group Forward should show a shallower slope to CS2 than to CS1 if they timed the 20-s CS2-US duration as temporal gradients to longer-duration CSs are associated with shallower slopes [e.g., 5].

The timing analyses overall indicate that all groups timed the CS1-US interval, but only Group Forward timed the CS2-US interval. However, they did not accurately time the CS2-US interval as the peak occurred at around 15 s, much earlier than the expected time of the peak at 20 s if they had accurately learned the CS2-US interval. While they did peak early, they were not simply transferring timing of the CS1-US interval, which would have led to steeper slopes and a median response time at 10 s. It is possible that the prolonged period of SOC training may have interfered with development of accurate and precise timing of the CS2 onset-US interval. This will be examined in Experiment 3 with a briefer SOC phase. First, Experiment 2 examined SOC with a new set of stimulus arrangements to further test the nature of the temporal integration process.

Experiment 2

While Experiment 1 partially supported the temporal coding hypothesis in terms of the effect of order of CS1 and CS2 presentations on the magnitude of second-order conditioning, there was no evidence of timing of CS2 onset-***US*** interval duration in the second-order phase. In the final phase, where all SOC trials ended in reinforcement, a more clearly defined peak function was observed, but only in Group Forward. There are two likely explanations of the pattern of results. It is possible that the CS2 onset-***US*** interval was learned, but not expressed during the second-order phase [see 20]. The presence of reinforcement in the final phase may have then allowed for expression of timing. Alternatively, SOC may have resulted in learning of the order of the stimuli, but not learning of the precise durations. The CS2 onset-US interval may have then been learned in the final phase through a separate process. The following two experiments will attempt to address these issues.

Experiment 2 involved the presentation of arrangements that would more fully test the application of the temporal coding hypothesis to both conditioning and timing of responding in SOC. Figure 5 illustrates the order and duration of the stimuli received by different groups of rats. The arrows below each procedural diagram indicate the intervals that could be learned directly in FOC (solid lines) and indirectly through temporal integration in SOC (dashed lines).

The first two groups (Trace and Forward) tested the possibility that the failure to time CS2 onset-***US*** in Group Forward during SOC may have been due to the lack of reinforcement on SOC trials. Previous research has indicated that reinforcement plays an important role in driving timing processes [21], so the lack of reinforcement on SOC trials may have resulted in a failure to engage temporal learning mechanisms. In prior research on temporal encoding by Cole et al. [4], the group that successfully established SOC received a trace condition, similar to the one diagrammed in Figure

5. This group could establish timing from CS onset-US (20 s) and from CS termination-US (10 s) in FOC [see 22 for an example of learning of these two durations in trace conditioning]. In SOC, CS2 occupied the 10-s period that was previously the trace interval. Thus, this group would merely have to transfer an interval that they had already been learned [23], and generalize this temporal learning to CS2. Assuming this is the case, then one would expect the trace group to show better SOC because of their shorter CS2 onset-***US*** interval, and they should display timing of the CS2 onset-***US*** interval during SOC because they already have acquired timing of that interval.

Two additional forward groups were also examined, both of which received partially-overlapping SOC compounds. These conditions were designed to further assess the failure of Group Simultaneous to display second-order conditioning in Experiment 1. Group Forward-Long received an arrangement that was comparable to Group Forward, except that CS2 remained on during CS1. The temporal map in Group Forward-Long should be identical to Group Forward, so any difference in SOC would most likely be due to overlap in the stimuli comprising the second-order compound. Group Forward-Short received a 20-s CS1, followed by the addition of a 10-s CS2 that occurred during the last half of CS1. This group provided a comparison with Group Trace. They would be expected to acquire a 20-s CS1 onset-US temporal interval in FOC, but would have to then learn a 10-s CS2 onset-***US*** interval in SOC. The temporal coding hypothesis would expect robust SOC in Group Forward-Short because of the short CS2 onset-***US*** interval, but there may be some disadvantage compared to Group Trace because the trace condition should have already established the 10-s trace duration in FOC, which could then be used to time from CS2 onset-***US***.

In addition, Group Forward-Short may experience a decrement due to the CS2 overlapping with CS1, consistent with Group Simultaneous in Experiment 1.

Method

Animals. The subjects were twenty-four experimentally-naïve male Sprague-Dawley rats (Harlan, UK). Housing and husbandry was the same as those in Experiment 1.

Apparatus. The apparatus was the same as that used in Experiment 1.

Procedure. Each animal was randomly assigned to one of four different groups (*n* = 6).

First-order conditioning (Sessions 1-8). All groups received eight sessions of FOC consisting of 32 reinforced trials per session. Each trial was separated by an ITI that was an exponentially distributed duration with a mean of 200 s and a minimum of 100 s. Different groups of rats received different FOC training, as shown in Figure 5. Group Trace received a 10-s light CS1 followed by a 10-s trace interval after which a single food pellet was delivered. Groups Forward and Forward-Long received a 10-s light CS1 followed by food delivery. Group Forward-Short received a 20-s light CS1 followed by food delivery.

Second-order conditioning (Sessions 9-18). SOC consisted of 10 sessions of 16 trials each and the ITI was the same as in FOC. A second stimulus, a 70-dB white noise, was introduced. The procedure received by each group is diagrammed in Figure 5. In Group Trace, CS2 was turned on following CS1 termination so that CS2 filled the trace interval and CS2 termination occurred at the expected time of US delivery. In Group Forward, CS2 immediately preceded CS1 so that CS2 termination coincided with CS1 onset; this condition was therefore identical to Group Forward in Experiment 1. In Group Forward-Long, CS2 was presented with CS1 in a forward manner, but CS2 was 20 s in duration. Consequently, there was a 10-s gap between CS2 onset and CS1 onset and the CSs terminated at the same time. Finally, in Group Forward-Short, there was a reversal of the procedure received by Group Forward-Long. A 10-s CS2 that co-terminated with CS1 was added and, consequently, CS1 was on for 10 s prior to CS2 onset. We tested for SOC by recording the magnitude and timing of responding during peak trials that consisted of non-reinforced noise presentations that lasted for 30 s.

The first two sessions of SOC (9-10) began with two FOC trials to maintain responding. As in Experiment 1, three trial types were randomly presented during the remainder of the session: two FOC trials, ten non-reinforced SOC trials, and two 30-s CS2 only non-reinforced peak trials. For the remaining eight sessions (11-18) the four FOC trials were delivered randomly during the session, the number of peak trials was increased to four, and the number of SOC trials was reduced to eight.

Reinforcement of the second-order compound (Sessions 19-43). During the first 10 sessions of this phase, the four groups were divided into two sub-groups. For each type of compound, one of the sub-groups received reinforced SOC+ trials in addition to the reinforced FOC trials; the other sub-group continued to receive nonreinforced SOC trials. Food delivery on SOC+ trials occurred at the same time relative to CS1 onset as in FOC training for each group (see dashed triangles in Figure 5). As in the previous phase, the CS2 only peak trials were not reinforced. The number of trials of each type was the same as in the last eight sessions of SOC. Following the initial 10 sessions in this phase, 15 sessions were delivered with both sub-groups of each compound type receiving reinforced SOC+ trials. Because of an error in the program received by the trace groups, which resulted in food delivery at the wrong time on SOC+ trials, they were excluded from the analyses in this phase.

Results and Discussion

First-order conditioning. First-order conditioning proceeded as expected. The rate of responding during the CS differed among the groups, $F(3,20) = 6.72$, $p = .003$, $\eta^2 = .50$. Tukey post-hoc tests isolated this difference to greater responding in Groups Forward (M = 27.2 $\pm$ 2.3 responses/min) and Forward-Long (M = 31.3 $\pm$ 3.5 responses/min) compared to Group Trace (M = 16.5 $\pm$ 1.8 responses/min), $p < .05$. This difference is most likely due to a trace conditioning deficit. Group Forward-Short, who received a 20-s CS duration, produced an intermediate response rate (M = 22.3 $\pm$ 1.9 responses/min) that did not differ from the other three groups. Although Group Trace demonstrated significantly lower rates of responding during the CS, they did produce substantial responding during the trace interval (M = 30.8 $\pm$ 3.4 responses/min). The response rate produced by Group Trace during the trace interval did not differ from the response rate produced by Groups Forward and Forward-Long during the 10-s CS.

Anticipatory timing also emerged, both during the CS and the trace interval (in Group Trace). These data are not shown here, but the timing functions are shown during FOC trials in the SOC phase, which were similar to the functions obtained by the end of FOC (see below).

Second-order conditioning.

Conditioning to CS2. The mean rate of responding during CS2 peak trials was examined for evidence of SOC (Figure 6). An ANOVA on the mean response rates with the variables of overlap (coding whether the CS1-CS2 compound overlapped or not) and CS1 onset-US duration (10 s for Groups Forward and Forward-Long and 20 s for Groups Trace and Forward-Short) indicated that the groups with overlapping compounds (Forward-Short and Forward-Long) performed significantly worse than the groups with non-overlapping compounds, $F(1,20) = 17.44$, $p < .001$, $\eta_p^2 = .47$. There was no effect of the CS1 onset – US duration ($p = .914$, $\eta_p^2 = .00$), nor any interaction of this variable with the overlap of the compounds ($p = .682$, $\eta_p^2 = .01$). Thus, it appears that overlapping compounds significantly impaired SOC, even though the resulting temporal maps in Groups Trace and Forward-Short should be highly similar and the maps in Groups Forward and Forward-Long should be the same. Overlapping SOC compounds appear to be highly detrimental to the development of SOC CRs to CS2. This finding is consistent with the failure of SOC in Group Simultaneous in Experiment 1.

Thus, Experiment 2 replicated the finding in Experiment 1 that forward-trained second-order compounds showed evidence for SOC. Furthermore, FOC followed by a backward compound also resulted in good SOC. Interestingly, Group Trace did not respond more than Group Forward. The temporal coding hypothesis [13, 14] predicts better SOC in Group Trace than Group Forward because temporal integration would lead to the expectation of a 10-s CS2 onset-***US*** interval in Group Trace compared to a 20-s CS2 onset-***US*** interval in Group Forward (Figure 5), and shorter intervals should result in better conditioning. In addition, Group Trace likely learned the 10-s CS1 termination-US interval, the interval filled by CS2, and would thus be expected to show an advantage over Group Forward. It is possible that the failure to observe a difference may be due to the choice of parameters, or to a lack of sensitivity in the procedure. On the other hand, the lack of difference between these groups may be due to the formation of an ordinal temporal map. This possibility will be explored further in the *General Discussion.*

Timing of CS1 and CS2 duration. Visual inspection of the pattern of responding indicated that response rates on FOC trials (Figure 7, dashed lines) increased over the course of CS1 and peaked slightly before the time of food delivery. In addition, Group Trace displayed a gradual increase in response rates during the light (0-10 s) followed by a steeper increase in response following light termination, which is consistent with learning of both CS1 onset-US and CS1 termination-US intervals [22].

Response rates on CS2 peak trials increased rapidly and reached their maximum in the first 2-3 s after noise onset. This was followed by a decline in responding over the remaining duration of CS2 and indicates that none of the groups were timing from noise onset to the expected time of US delivery during the peak trials. The expected time of the peak, assuming learning of an accurate temporal map, is marked by an arrow in each panel of the figure, and this time was accompanied by a low rate of responding in all groups. There was a general trend towards Group Forward displaying a later peak compared to Group Trace, but the peaks were not appropriately timed. If the two groups had learned the CS2 onset-***US*** interval, Group Forward should have peaked 10 s later than Group Trace, but instead their peak appeared 3-5 s later. Moreover, Group Trace did not display transfer of timing from the trace interval to CS2. Although they already had acquired interval timing of the 10-s interval following CS1, generalization of temporal learning did not occur. It thus appears that reinforcement may be necessary for expression of timing in SOC even when there are temporal representations from FOC that could be transferred to SOC.

Reinforcement of the second-order compound. Because we again found no convincing evidence for timing of the noise during SOC, an additional phase was added during which half the rats in the four groups received reinforced SOC+ trials. The other half of the rats continued to receive nonreinforced SOC trials. This manipulation was conducted to check that the emergence of timing under reinforcement in Experiment 1 was not due simply to further training. Figure 8 displays the timing functions for each group of rats during the CS2 peak trials. Separate functions are displayed for the first 10 sessions when the reinforced groups (solid lines) received reinforcement and the other groups did not (dashed lines; left column), and the last 15 sessions during which all groups received reinforced SOC trials (right column). The trace groups are not presented because of an error in the program (see *Methods*); these rats displayed little or no responding because of the error.

As seen in the figure, the Forward and Forward-Long sub-groups that received reinforcement (heavy solid lines) displayed peaked functions, but the sub-groups that received nonreinforced SOC trials (thin dashed lines) displayed relatively flat functions. Both the sub-groups with the Forward-Short compounds displayed flat and noisy functions, indicating that reinforcement had no effect on responding in these groups. During the following 15 sessions (right column), when all groups received reinforced SOC trials, both sub-groups of rats receiving Forward and Forward-Long compounds now displayed peaked functions, but the Forward-Short groups continued to display relatively constant and noisy functions. An ANOVA was conducted on the slopes for the first 10 s and 20 s of the peak trials with the variables of group (Forward, Forward-Short, and Forward-Long), reinforced (reinforcement vs. nonreinforcement of SOC trials) and session (Sessions 29-43 vs. Sessions 44-58). For the 10-s slopes, the slopes were steeper during Sessions 44-58 compared to Sessions 29-43, $F(1,12) = 9.30$, $p = .010$, $\eta_p^2 = .44$, reflecting the influence of further training on the expression of timing. Relatedly, there was an overall effect of reinforced training in that slopes were steeper under reinforcement than non-reinforcement, $F(1,12) = 11.06$, $p = .006$, $\eta_p^2 = .48$. There was also a Reinforced × Session interaction, $F(1,12) = 5.14$, $p = .043$, $\eta_p^2 = .30$ which was due to steeper slopes in the later sessions for the rats that received non-reinforcement in the early sessions. Finally, there was a group main effect, $F(2,12) = 18.38$, p = .001, $\eta_p^2 = .54$, that was due to groups Forward and Forward-Long displaying steeper slopes than Group Forward-Short. There was a non-significant trend towards an effect of Group × Session ($p = .054$, $\eta_p^2 = .39$), but no indication of any effect of Group × Reinforced ($p = .240$, $\eta_p^2 = .21$) or Group × Reinforced × Session ($p = .306$, $\eta_p^2 = .18$).

For the 20-s slopes, there was a Group main effect, $F(2,12) = 8.14$, $p = .006$, $\eta_p^2 = .58$ that was due to Group Forward-Long showing a significantly steeper slope overall than group Forward-Short. There also was a Group × Session interaction, $F(1,12) = 6.25$, $p = .014$, $\eta_p^2 = .51$ that was due to increased slopes in Groups Forward and Forward-Long over sessions, but not in Group Forward-Short. There was a non-significant trend towards increased slopes as a function of Session, ($p = .053$, $\eta_p^2 = .28$), but no significant effects of reinforced ($p = .181$, $\eta_p^2 = .14$), Group × Reinforced ($p = .348$, $\eta_p^2 = .16$), Reinforced × Session ($p = .336$, $\eta_p^2 = .08$), or Group × Reinforced × Session ($p = .466$, $\eta_p^2 = .12$),

Thus, is seems that only Groups Forward and Forward-Long showed substantial temporal control and this was much more pronounced under reinforcement. To assess timing accuracy in these groups under the conditions of reinforcement, the time of the median response was computed and compared to 10 s and 20 s using one-sample t-tests. Group Forward displayed median response times (13.7 $\pm$ 1.1 s) that were significantly later than 10 s, $t(5) = 3.29$, $p = .022$, and were significantly earlier than 20 s, $t(5) = -5.68$, $p = .002$. Group Forward-Long also had median response times (16.8 $\pm$ .6) that were later than 10 s, $t(5) = 11.4$, $p < .001$, and earlier than 20 s, $t(5) = -5.27$, $p = .003$.

Thus, it appears that the peak functions that emerged in the Forward group during the reinforced SOC phase in Experiment 1 were observed again, and that these were a product of reinforcement. It also appears that successful SOC promoted the expression of timing under reinforcement, as Groups Forward and Forward-Long exhibited timing and SOC. However, both groups showed peaks that were earlier than expected if they had accurately determined the CS2-US interval, but also were later than the CS1-US interval. This indicates that they were not likely transferring the timing of CS1-US to the timing of CS2-US. It is interesting that Group Forward-Short did not display timing. This group had complete overlap of CS2 with CS1. It is possible that the degree of overlap is more apparent in the effects on timing, whereas overlap in general is detrimental to CR emergence. In Group Forward-Long, CS2 supplied unique temporal information during Seconds 11-20 prior to the expected time of the US, whereas in Group Forward-Short, CS2 provided redundant temporal information.

Experiment 3

According to the temporal coding hypothesis, SOC should result in temporal integration of the order that stimuli are presented in, and the temporal relationship between the times of these events. Taking this prediction at face value, SOC should enhance timing performance in subsequently reinforced second-order compounds. The purpose of the present experiment was to investigate whether SOC exposure would facilitate timing of the CS2 onset-US interval during reinforced SOC compounds relative to control groups that did not receive SOC. The trace and forward arrangements from Experiment 2 were the focus of these manipulations because they were the most effective in producing SOC. The SOC phase was briefer than in the previous two experiments to minimize the development of conditioned inhibition because it has been shown that a determinant of conditioned inhibition is the number of nonreinforced second-order trials [6, 24]. Conditioned inhibition may have interfered with timing expression in SOC in the previous studies, so the shorter duration of training should help to combat this effect.

Method

Animals. The experimental subjects were twenty-four experimentally-naïve male Sprague-Dawley rats (Harlan, UK). Housing and husbandry was the same as in the previous two experiments.

Apparatus. The apparatus was the same as that used in Experiments 1 and 2.

Procedure. Each animal was randomly assigned to one of four different groups (n = 6).

First-order conditioning (Sessions 1-17). All rats received 17 sessions of FOC that were identical to Experiment 2 (see Figure 5). Two groups of rats received a standard delay conditioning procedure consisting of a 10-s light stimulus followed by the delivery of a single 45-mg Noyes food pellet (forward groups; see Forward group in Figure 5). The remaining two groups received training on a trace conditioning procedure that consisted of a 10-s light stimulus followed by a 10-s trace interval followed by food (trace groups; see Trace group in Figure 5). These conditions are identical to the trace and forward groups from Experiment 2.

Second-order conditioning (Sessions 18-19). Following FOC, two sessions of SOC were delivered to Groups Forward and Trace. The control groups, (Forward-Control and Trace-Control) were placed in the conditioning chambers, but received no stimulus exposures. The SOC phase was delivered to Groups Forward and Trace in a manner identical to the first two sessions of SOC in Experiment 2.

Reinforcement of the second-order compound (Sessions 20-31). Twelve sessions of reinforced second-order compound training were given to all groups following SOC. The procedure was identical to SOC except that all SOC trials ended in reinforcement, and the four FOC trials were replaced with reinforced SOC trials.

Results and Discussion

First-order conditioning. The mean response rates for the forward- and trace-conditioned groups were averaged over the seventeen sessions of FOC. The mean response rates during the CS were 10.2 $\pm$ 1.0, 8.6 $\pm$ 1.0, 31.1 $\pm$ 4.1, and 31.8 $\pm$ 3.2 responses/min for Group Trace, Trace-Control, Forward, and Forward-Control, respectively. An ANOVA on these rates revealed a significant effect of procedure (trace vs. forward), $F(1,20) = 65.73$, $p < .001$, $\eta_p^2 = .77$; this result is the expected trace deficit in responding. There was no effect of condition (experimental versus control; $p = .869$, $\eta_p^2 = .00$) or any interaction of condition and procedure ($p = .690$, $\eta_p^2 = .01$), indicating that the experimental and control groups did not differ during FOC. A comparison of the response rates from the 10-s trace interval in the trace groups (Trace = 29.9 $\pm$ 4.9, Trace-Control = 33.1 $\pm$ 4.8 responses/min) and the response rates during the 10-s CS in the forward groups (see means above) revealed that there was no effect of procedure ($p = .999$, $\eta_p^2 = .00$), no effect of condition ($p = .653$, $\eta_p^2 = .01$) or an interaction ($p = .763$, $\eta_p^2 = .01$). This result is consistent with the notion that the trace groups learned the 10-s trace interval, and is indicative of substantial control by the trace interval over responding.

All groups displayed an increase in responding from CS onset and the maximum rate of responding was reached prior to US delivery; these data are not shown here, but Figure 7 displays similar functions obtained during the reinforcement of second-order compound phase of Experiment 2.

Second-order conditioning. The mean response rates produced by the two groups (Forward and Trace) did not differ. The results yield further evidence that the trace arrangement failed to produce better conditioning than the forward arrangement, despite having a more efficacious temporal relationship with food.

Reinforcement of the second-order compound. The timing functions were examined during reinforced SOC compound training to look for any evidence of an effect of SOC on the emergence of timing (see Figure 9). The peak functions for the first two sessions (20-21, thin solid lines) and the remaining 10 sessions (22-31, thick solid lines) are presented separately for each group. An examination of the peak trials revealed that the response rate functions in the forward groups displayed a peak at around 10-15 s, whereas the trace groups displayed an early peak followed by a gradual decline in responding. With further training, the forward groups displayed a more defined peak in responding, but the trace groups continued to display noisy, flat functions. This was confirmed by ANOVA where there was an effect of procedure (trace vs. forward) at both 10 s, $F(1,19) = 17.43$, $p = .001$, $\eta_p^2 = .48$, and 20 s, $F(1,19) = 13.88$, $p = .044$, $\eta_p^2 = .20$, that was due to steeper slopes in the Forward groups. There was no effect of condition (experimental vs. control; $p\text{s} \geq .336$, $\eta_p^2 \leq .05$) or any Procedure × Condition interaction ($p\text{s} \geq .670$, $\eta_p^2 \leq .01$). As there was no effect of condition, we conducted one-sample t-tests on the data collapsed across condition and compared the slopes to zero. Group Forward showed significant positive slopes at 10 s, $t(11) = 6.87$, $p < .001$, but not at 20 s, $t(11) = 1.94$, $p = .08$. There was no evidence for temporal control for Group Trace at either 10 or 20 s as their slopes did not differ from zero ($p\text{s} \geq .22$). We additionally examined the median response times for Group Forward (over sessions 22-31) to assess their timing accuracy. The median response times produced by Group Forward (12.8 + .4 s) were significantly later than

10 s, $t(11) = 6.99$, $p < .001$ and were also significantly earlier than 20 s, $t(11) = -17.69$, $p < .001$. Thus, we replicated the findings of Experiments 1 and 2 with respect to Group Forward.

There are four main trends to the peak functions: (1) the forward groups peaked later than in the trace groups; (2) only the forward groups produced a defined peak function indicative of temporal control; (3) the forward groups peaked before the expected time (20 s) based in temporal map integration (marked by arrows in the figure), but also peaked later than 10 s indicating that they did not simply transfer CS1-US timing to CS2; and (4) there were no striking effects of SOC exposure on the peak functions (Trace vs. Trace-Control, Forward vs. Forward-Control).

Considering these additional findings, it appears that the brief SOC exposure did not facilitate learning of the CS2 onset-***US*** interval. If such learning were to occur, one might expect to be more likely to observe it in the trace arrangement where CS2 filled the trace interval. In this case, CS2 filled a position in the temporal map that was already learned in FOC. However, this group displayed weaker evidence of timing than Group Forward.

General Discussion

In terms of the strength of SOC, the pattern of results shown here were partially consistent with the temporal coding hypothesis [13]. Figure 10 displays the different arrangements and the degree of SOC that was observed. There are two main trends to be seen in the figure. First, when CS2 was in a forward relationship with the expected time of the US that was established in FOC, SOC was observed. This can be seen in the Forward and Trace groups. On the other hand, when CS2 was in a backward or unpaired relationship with the US, SOC was weak (Backward and Unpaired groups). The unpaired condition assesses the degree of SOC due to generalization between CS1 and CS2, in the absence of any effective temporal cues; Group Backward did not demonstrate any advantage over Group Unpaired in SOC indicating the that temporal coincidence of CS2 and CS1 was not sufficient to promote CRs. These findings are consistent with the temporal coding hypothesis. The trace and backward groups are comparable with the trace and delay groups employed by Cole et al. [4], in which the trace group demonstrated superior SOC. Although these two groups were not directly compared in the same experiment here, the general pattern of results is consistent with Cole et al.'s previous finding and therefore represents a confirmation of these results in an appetitive conditioning procedure. The comparison between trace and backward conditions is interesting because these two groups both received a backward CS1→CS2 pairing in SOC, so the difference between them was not due to the nature of the second-order compound.

An additional factor that is not predicted by the temporal coding hypothesis is that when CS1 and CS2 overlapped in time, SOC was impaired. This can be seen by examining the Simultaneous, Forward-Long, and Forward-Short groups. These results might be explained by a generalization decrement, which could lead to performance failures to CS2 due to testing of CS2 on its own [see 12]. An additional possible source of the poor SOC in the overlapping compounds may be the development of conditioned inhibition. Stout, Escobar, and Miller [6] reported that conditioned inhibition, as assessed by summation and retardation tests, developed most rapidly during SOC with simultaneous compounds. On the other hand, serial compounds led to much more robust SOC than simultaneous or overlapping compounds. Although conditioned inhibition was not directly tested here, the pattern of results is consistent with the development of inhibition, which would hinder SOC.

Future studies should incorporate explicit tests for conditioned inhibition to assess this possibility.

Another finding that was not supported by the temporal coding hypothesis was the comparison between forward and trace conditions in Experiment 2. Here, it was expected that the trace condition should yield better performance than the forward condition because the CS2 onset-***US*** in Group Trace was only 10 s, in comparison to a 20-s interval in Group Forward. However, the failure may be due to an insensitivity in the parameters used here, as the precise timing of CS1 and CS2 have previously been found to produce differences in SOC in the rabbit nictitating membrane preparation. Kehoe et al. [10] examined the effect of CS2 onset → CS1 onset and CS1 onset → US intervals on the magnitude of responding to CS2 in a forward SOC arrangement. The percentage of CRs was a function of both interval durations, indicating a sensitivity to the timing of events in SOC on CR magnitude.

While the magnitude of second-order responding did generally concur with the temporal coding hypothesis, there was no evidence of timing of the CS2 onset-***US*** interval during SOC. The timing functions in SOC peaked early and then gradually fell as a function of time since CS2 onset. In general, the temporal gradients in SOC did not support the notion of a fully featured temporal map having been formed. This is consistent with the two-process framework proposed by Kirkpatrick and Balsam [15] in which temporal maps are reliant only on event coincidence and order information, whereas timing is reliant on a separate delay processing system. However, there is evidence in the literature to suggest that at least simple timing may occur in temporal map formation. Specifically, Leising, Sawa and Blaisdell [25] presented an overlapping CS1-CS2 compound in sensory pre-conditioning in which CS2 was 60 s in duration and CS1 was 10 s in duration. CS1 occurred either early or late in CS2 in separate groups, and then was later presented in a simultaneous compound with the US. They found that the time of occurrence of CS1 influenced the time of responding during CS2 test trials, although precise timing was not observed. One important factor may be the use of a sensory pre-conditioning procedure for temporal map formation in their procedure, which may have potentially reduced conditioned inhibition formation. Future research should directly compare SOC and sensory pre-conditioning procedures to determine whether this is the case.

An interesting aspect of the SOC data is the apparent evidence of conditioning in the absence of overt timing behavior. There have been several observations indicating that timing and conditioning normally emerge together [e.g., 5, 26-28], but these previous studies were conducted under conditions where the CS was consistently followed by a US. On the other hand, an examination of responding under extinction has revealed evidence of separation of conditioning and timing, with extinction resulting in a reduction in the rate of response, but a maintenance of timing accuracy [29, 30]. Thus, it appears that altering the probability of reinforcement may allow for a dissociation of timing and conditioning processes, as was the case in the present experiment. Although timing and conditioning may co-occur, these processes appear to be at least partially independent. This is consistent with the modular theory of timing and conditioning [31] which proposes that updates in the conditioning module are independent from the timing module and are influenced by both reinforcement and nonreinforcement. The conditioning module controls the rate of response. Updates to the timing module only occur during reinforcement and influence the pattern of responding. Although the modular theory does not contain any mechanism for temporal map formation, the theory predicts the separate effects of reinforcement on timing and conditioning that were observed here.

Although there was no evidence of timing during SOC, temporal gradients emerged when the second-order compound was reinforced. This was observed in all three experiments in groups that demonstrated SOC except for Group Trace. The failure of Group Trace to demonstrate adequate timing may indicate an important role for event coincidence in guiding timing processes. Group Trace was the only group where CS offset did not coincide with US occurrence during FOC. In addition, groups that did not demonstrate SOC also did not acquire temporal gradients during the reinforced compound phase. For example, Group Forward-Short, which had a forward CS2-US relationship, did not demonstrate robust SOC (Figure 6), and did not acquire a temporal gradient during the reinforced compound phase (Figure 7). However, Group Forward-Long did not demonstrate SOC (Figure 6) but then did later demonstrate timing (Figure 8) when the SOC compound was reinforced. Thus, while SOC expression and timing expression were correlated in most conditions, Group Forward-Long was an exception. The change in the gradients under reinforced compounds did not appear to be due simply to further training as only the reinforced sub-groups in Experiment 2 demonstrated peaked functions. Due to the paucity of evidence in the literature, one can only speculate that the groups that failed to display second-order responding may have experienced substantial inhibitory learning, thereby prohibiting any subsequent acquisition.

Experiment 3 assessed whether SOC would facilitate the acquisition of timing under reinforced compounds in the forward and trace conditions. There was no indication of any facilitation. These results, coupled with the failure to observe timing during the second-order phase in Experiments 1 and 2 suggest that learning of the precise durations between events did not occur.

The combined results of the conditioning and timing measures suggest that a temporal map may have been formed during SOC, but that the map may be reliant on temporal coincidence and order rather than interval timing processes, which is inconsistent with the temporal coding hypothesis. As an example of how event order and coincidence could result in a temporal map in the trace condition, FOC would result in learning CS1 on → CS1 off → US. In the second-order phase, the animal would then learn CS1 on → CS1 off/CS2 on → CS2 off. If the animal could invoke a representation of the US occurring sometime after CS1 termination, then this would lead to a map that contained CS2 onset prior to US occurrence. An ordinal map of this sort would allow for a differentiation of the order of events, but not precise timing. As another example, Group Forward would learn the series CS1 on → CS1 off/US and then learn the series CS2 on → CS2 off/CS1 on → CS1 off. The animal would simply have to remember that the US was coincident with CS1 termination. Here, as in the trace group, CS2 onset occurs prior to the memory of the US occurrence and would lead to good SOC, but without precise knowledge of the CS2 onset–***US*** interval, this would be no better than the trace condition. This explanation would also account for the poor second-order conditioning observed in a condition such as group Backward in Experiment 1. This group would acquire CS1 on → CS1 off/US and then acquire CS1 on → CS1 off/CS2 on → CS2 off. Here CS2 onset is coincident with CS1 offset, which was previously coincident with the US. Thus, CS2 onset does not precede the remembered time of occurrence of the US.

An ordinal temporal map does not require encoding of precise durations, and thus may explain why the trace and forward groups did not differ in the magnitude of SOC. In addition, an ordinal map does not lead to an expectation of timing from CS2 onset because the organism expects the US soon, but does not know the precise time when the US should occur. A series of experiments by Williams and Hurlburt [9] yielded results that are consistent with an ordinal map. First, they found that there was no difference between forward CS2-CS1-***US*** condition and backward CS2-***US***-CS1 in the magnitude of SOC even though CS2 in the backward condition was in closer temporal proximity to the expected time of the US. This result is much like the present finding of similar SOC in trace and forward conditions. Second, when a 3-s gap was imposed in the backward condition (e.g., CS2-***US***--CS1), SOC was disrupted. This finding implies that the coincidence of event onset/offset, such as CS2 offset and CS1 onset or US and CS1 onset, is necessary for the formation of SOC.

It is possible that an ordinal temporal map could be established via an associative chain, an idea that has been previously applied to the analysis of SOC [e.g., 32, 33]. Two different stimuli may become associated through the coincident occurrence of events (e.g., CS1 becomes associated with CS2 if a CS1 event transition coincides with a CS2 event transition). This additional assumption allows the associative chain mechanism to encode the order of events. For example, it allows for a determination that CS2 onset occurs prior to US occurrence in Group Trace.

While the notion of an ordinal temporal map corresponds with the general pattern of the timing and conditioning results, the rats acquired temporal knowledge in FOC. Why would this information fail to influence SOC? One possible answer stems from the effects of nonreinforcement on timing processes in SOC. It was apparent that reinforcement was necessary for the expression of timing in all three experiments, leading to the conclusion that reinforcement may be critical for driving timing processes. It does appear that reinforcement is important for the expression of CRs [15, 20]. In addition, reinforcement plays an important role in resetting the internal clock [21], and timing is more accurate when initiated by reinforcement [34].

It seems clear from the present results that reinforcement was a necessary condition for the observation of timing behavior, and this may have been due to a failure to learn the specific CS2 onset-***US*** interval during SOC.

Overall, the results of the present study are consistent with the temporal encoding hypothesis, but with the caveat that the temporal map does not contain fully featured timing information. These findings have important implications for understating the role of timing in the conditioning process and vice versa. These processes may be separate, but still nonetheless may interact under normal conditioning arrangements where reinforcement is prevalent. Further research should aim to better understand the nature of these two processes, their interaction, and how reinforcement may affect the expression of CRs and CR timing.

References

1. Rescorla, R.A., *Second-order conditioning.* Second order conditioning., 1980.
2. Ward-Robinson, J., *An analysis of second-order autoshaping.* Learning and Motivation, 2004. **35**: p. 1-21.
3. Barnet, R.C., R.P. Cole, and R.R. Miller, *Temporal integration in second-order conditioning and sensory preconditioning.* Animal Learning & Behavior, 1997. **25**(2): p. 221-233.
4. Cole, R.P., R.C. Barnet, and R.R. Miller, *Temporal encoding in trace conditioning.* Animal Learning & Behavior, 1995. **23**(2): p. 144-153.
5. Kirkpatrick, K. and R.M. Church, *Independent effects of stimulus and cycle duration in conditioning: the role of timing processes.* Animal Learning & Behavior, 2000. **28**: p. 373-388.
6. Stout, S., M. Escobar, and R.R. Miller, *Trial number and compound stimuli temporal relationship as joint determinants of second-order conditioning and conditioned inhibition.* Learning & Behavior, 2004. **32**(2): p. 230-239.
7. Rescorla, R.A., *Simultaneous second-order conditioning produces S-S learning in conditioned suppression.* Journal of Experimental Psychology: Animal Behavior Processes, 1982. **8**(1): p. 23-32.
8. Mowrer, R.R., D.E. Krug, and S.B. Klein, *Backward second-order conditioning in flavor aversion learning.* Psychological Record, 1988. **38**(2): p. 259-269.
9. Williams, D.A., & Hurlburt, J L, *Mechanisms of second-order conditioning with a backward conditioned stimulus.* Journal of Experimental Psychology: Animal Behavior Processes, 2000. **26**(3): p. 340-351.
10. Kehoe, E.J., A.M. Feyer, and J.L. Moses, *Second-order conditioning of the rabbit's nictitating membrane response as a function of the CS2-CS1 and CS1-US intervals.* Animal Learning & Behavior, 1981. **9**(3): p. 304-315.
11. Kim, S.D., et al., *Conditioned stimulus determinants of conditioned response form in Pavlovian fear conditioning.* Journal of Experimental Psychology: Animal Behavior Processes, 1996. **22**(1): p. 87-104.
12. Barnet, R.C., H.M. Arnold, and R.R. Miller, *Simultaneous conditioning demonstrated in second-order conditioning: evidence for similar associative structure in forward and simultaneous conditioning.* Learning and Motivation, 1991. **22**(253-268).
13. Savastano, H.I. and R.R. Miller, *Time as content in Pavlovian conditioning.* Behavioural Processes, 1998. **44**: p. 147-162.
14. Arcediano, F. and R.R. Miller, *Some constraints for models of timing: a temporal coding hypothesis perspective.* Learning and Motivation, 2002. **33**(1): p. 105-123.
15. Kirkpatrick, K. and P.D. Balsam, *Associative learning and timing.* Current Opinion in Behavioral Sciences, 2016. **8**: p. 181-185.
16. Tatham, T.A. and K.R. Zurn, *The Med-PC experimental apparatus programming system.* Behavior Research Methods, Instruments, and Computers, 1989. **21**(2): p. 294-302.
17. Hatfield, T., et al., *Neurotoxic lesions of basolateral, but not central, amygdala interfere with pavlovian second-order conditioning and reinforcer devaluation effects.* Journal of Neuroscience, 1996. **16**(16): p. 5256-5265.
18. Roberts, S., *Isolation of an internal clock.* Journal of Experimental Psychology: Animal Behavior Processes, 1981. **7**(3): p. 242-268.

19. Guilhardi, P. and R.M. Church, *Measures of temporal discrimination in fixed-interval performance: A case study in archiving data.* Behavior Research Methods, Instruments & Computers, 2004. **36**(4): p. 661-669.
20. Ohyama, T. and M.D. Mauk, *Latent acquisition of timed responses in cerebellar cortex.* The Journal of Neuroscience, 2001. **21**(2): p. 682-690.
21. Church, R.M., W.H. Meck, and J. Gibbon, *Application of scalar timing theory to individual trials.* Journal of Experimental Psychology: Animal Behavior Processes, 1994. **20**(2): p. 135-155.
22. Desmond, J.E. and J.W. Moore, *Altering the synchrony of stimulus trace processes: Tests of a neural-network model.* Biological Cybernetics, 1991. **65**: p. 161-169.
23. Bonardi, C. and D.J. Jennings, *The effects of stimulus distribution form during trace conditioning.* Quarterly Journal of Experimental Psychology, 2017.
24. Yin, H., R.C. Barnet, and R.R. Miller, *Second-order conditioning and Pavlovian conditioned inhibition: Operational similarities and differences.* Journal of Experimental Psychology: Animal Behavior Processes, 1994. **20**(4): p. 419-428.
25. Leising, K.J., K. Sawa, and A.P. Blaisdell, *Temporal integration in Pavlovian appetitive conditioning in rats.* Learning & Behavior, 2007. **35**(1): p. 11-18.
26. Balsam, P.D., M.R. Drew, and C. Yang, *Timing at the start of associative learning.* Learning and Motivation, 2002. **33**: p. 141-155.
27. Maes, J.R. and J.M. Vossen, *One-trial aversive conditioning to contextual cues: Effects of time of shock presentation on freezing during conditioning and testing.* Bulletin of the Psychonomic Society, 1992. **30**(5): p. 403-406.
28. Bevins, R.A. and J.J.B. Ayres, *One-trial context fear conditioning as a function of the interstimulus interval.* Animal Learning & Behavior, 1995. **23**(4): p. 400-410.
29. Bonardi, C., B. Brilot, and D.J. Jennings, *Learning about the CS during latent inhibition: Preexposure enhances temporal control.* Journal of Experimental Psychology: Animal Learning and Cognition, 2016. **42**(2): p. 187-199.
30. Ohyama, T., et al., *Temporal control during maintenance and extinction of conditioned keypecking in ring doves.* Animal Learning & Behavior, 1999. **27**(1): p. 89-98.
31. Guilhardi, P., L. Yi, and R.M. Church, *A modular theory of learning and performance.* Psychonomic Bulletin & Review, 2007. **14**(4): p. 543-559.
32. Mackintosh, N.J., *The psychology of animal learning*. 1974, London: Academic Press.
33. Wagner, A.R. and S.E. Brandon, *Evolution of a structured connectionist model of Pavlovian conditioning (AESOP)*, in *Contemporary learning theories: Pavlovian conditioning and the status of traditional learning theory*, S.B. Klein and R.R. Mowrer, Editors. 1989, Lawrence Erlbaum Associates: New Jersey. p. 149-189.
34. Spetch, M.L. and D.M. Wilkie, *Duration discrimination is better with food access as the signal than with light as the signal.* Learning and Motivation, 1981. **12**: p. 40-64.

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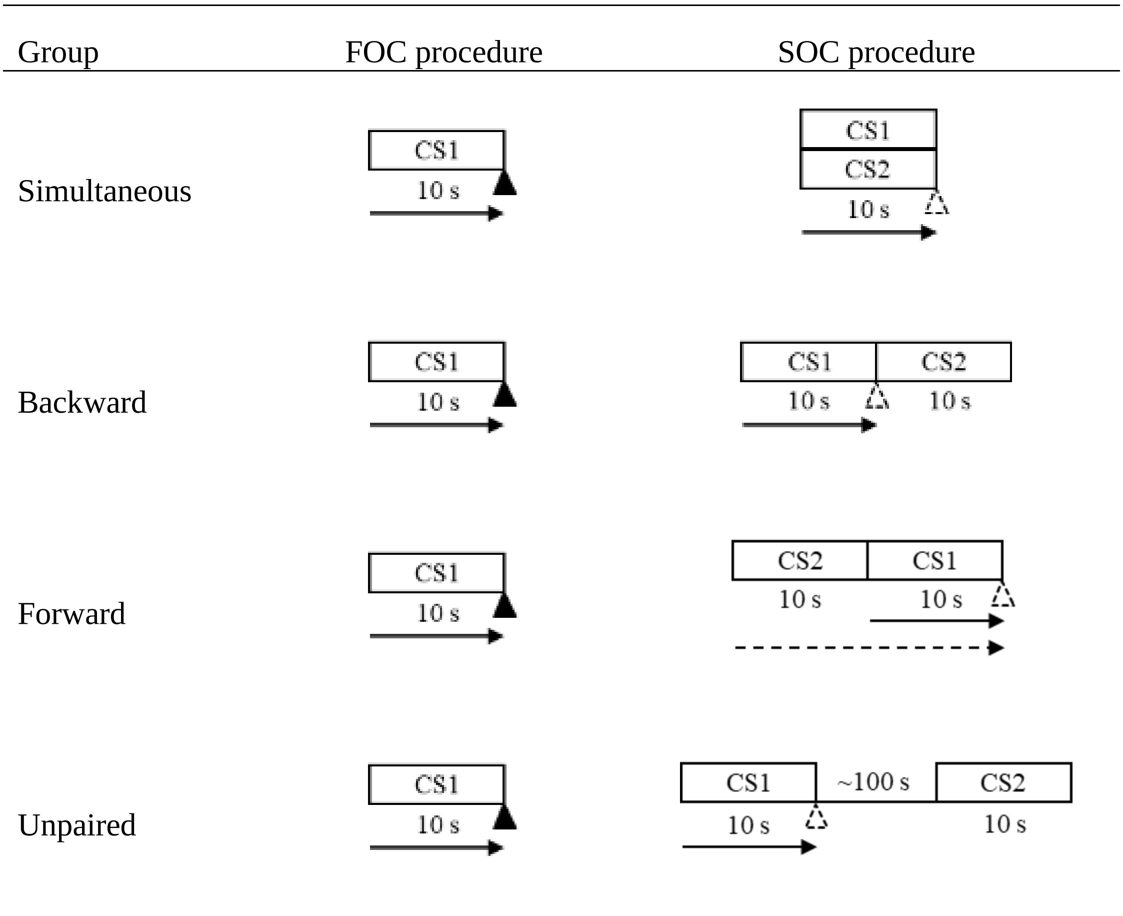

Figure 1. The procedure received by each group in Experiment 1 during first-order (FOC) and second-order conditioning (SOC) phases. The CSs are represented by rectangles, and food delivery on FOC trials by filled triangles. Food delivery on reinforced SOC+ trials is indicated as a dashed open triangle. The arrows below each procedure indicate the forward temporal intervals that could be learned directly in first-order conditioning (solid lines) and indirectly through temporal integration during second-order conditioning (dashed lines).

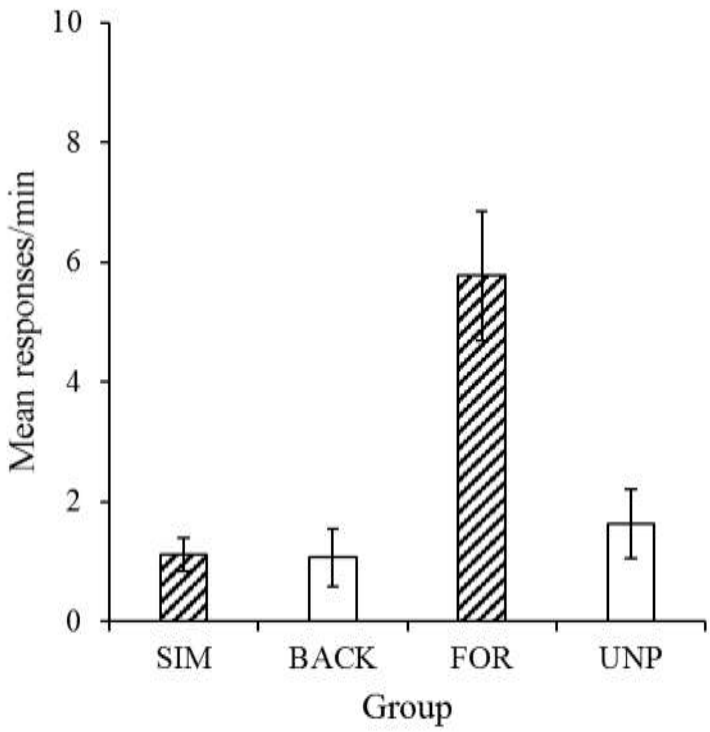

Figure 2. The mean rate of responding during the first 10 s of CS2 peak trials for the four different groups, collapsed across sessions in Experiment 1. The two groups that would be expected to learn the CS2-***US*** interval in SOC are denoted with hatched bars. The error bars are ± one standard error of the mean. SIM = Group Simultaneous; BACK = Group Backward; FOR = Group Forward; UNP = Group Unpaired.

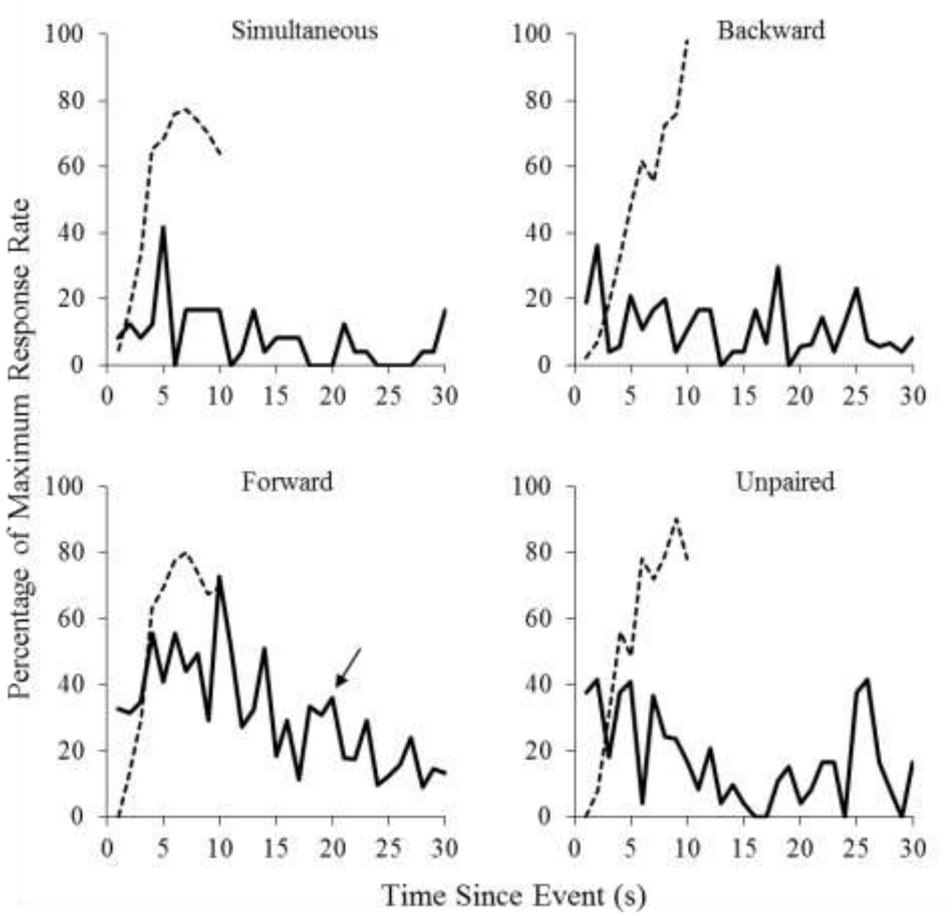

Figure 3. The percentage of the maximum response rate as a function of time since either CS1 onset on FOC trials (dashed lines), or CS2 onset on peak trials (solid lines) for each of the four groups during second-order conditioning in Experiment 1. The expected time of US delivery, assuming the formation of an integrated temporal map, is denoted by an arrow for groups with forward CS2 onset-***US*** intervals. The CS2 functions should peak at the time denoted by the arrow if the rats acquired an accurate CS2 onset-***US*** representation.

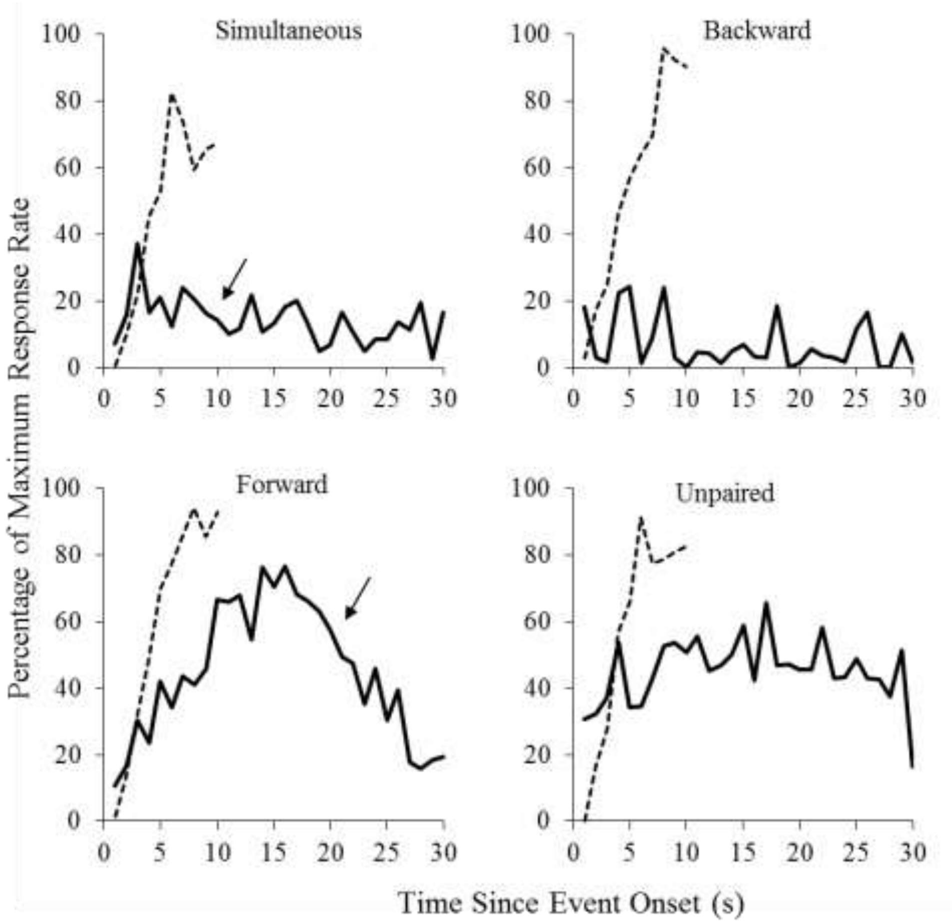

Figure 4. The percentage of the maximum response rate as a function of time since either CS 1 onset on FOC trials (dashed lines) or CS2 onset on peak trials (solid lines) for each of the four groups during reinforced compound training in Experiment 1. The expected time of US delivery relative to CS2 onset assuming the formation of an integrated temporal map, is denoted by an arrow for groups with forward CS2 onset-***US*** intervals.

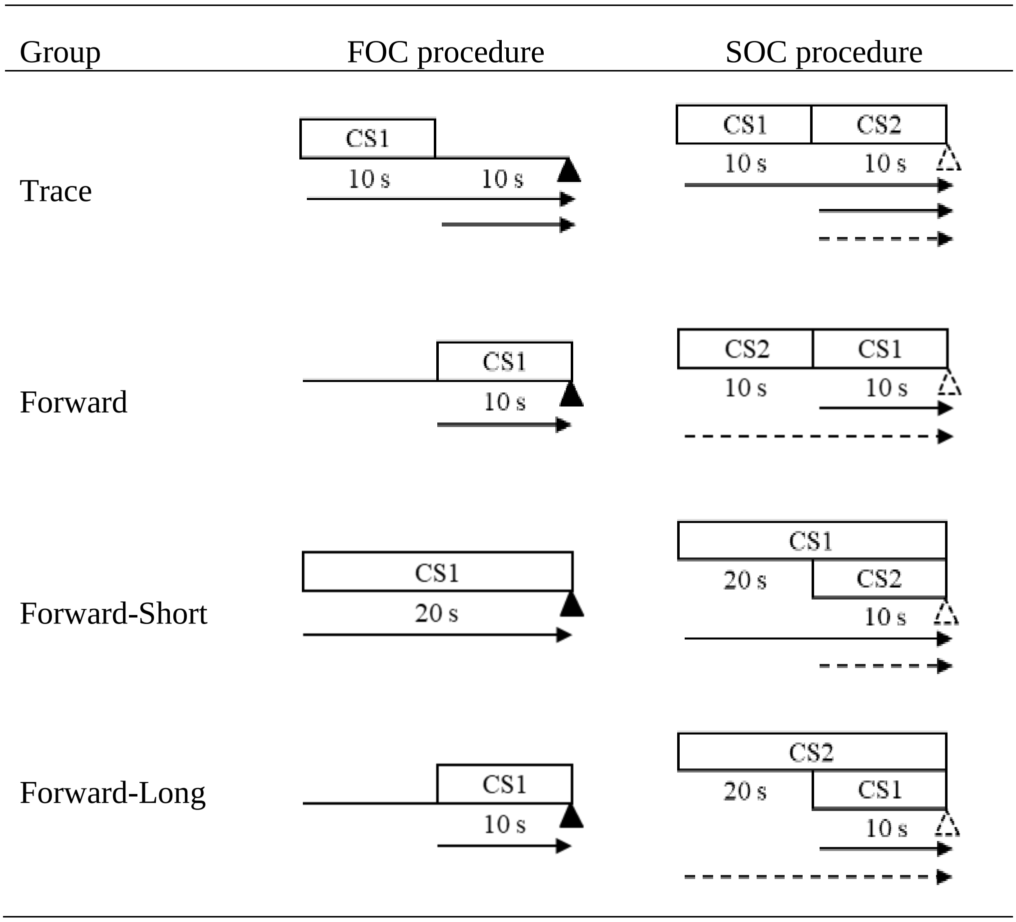

Figure 5. The procedure received by each group in Experiment 2 during first-order (FOC) and second-order conditioning (SOC) phases. The CSs are represented by rectangles, and food delivery on FOC trials by filled triangles. Food delivery on reinforced SOC+ trials is indicated as a dashed open triangle. The arrows below each procedure indicate the forward temporal intervals that could be learned directly in first-order conditioning (solid lines) and indirectly through temporal integration during second-order conditioning (dashed lines).

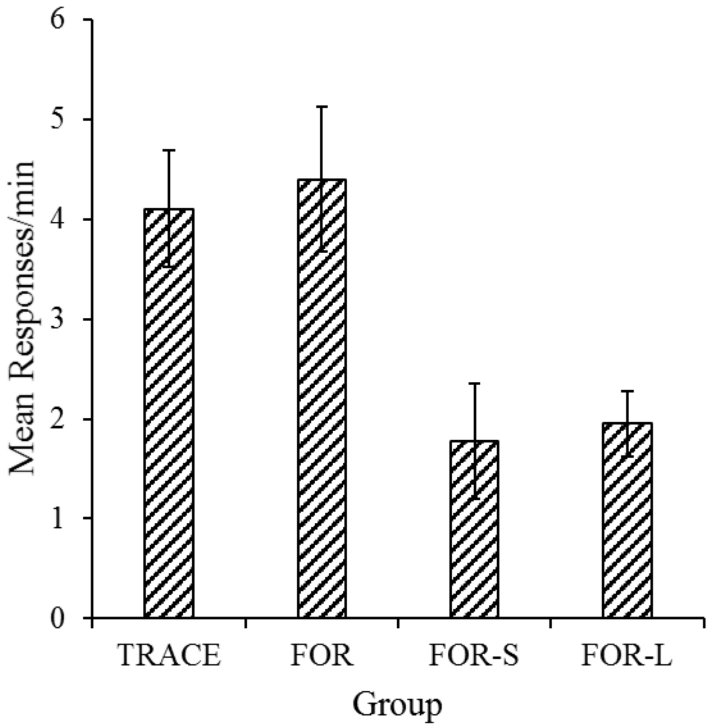

Figure 6. The mean rate of responding during the first 10 s of CS2 peak trials for the four different groups, collapsed across sessions in Experiment 2. The error bars are $\pm$ one standard error of the mean. TRACE = Group Trace, FOR = Group Forward, FOR-S = Group Forward-Short, FOR-L = Group Forward-Long.

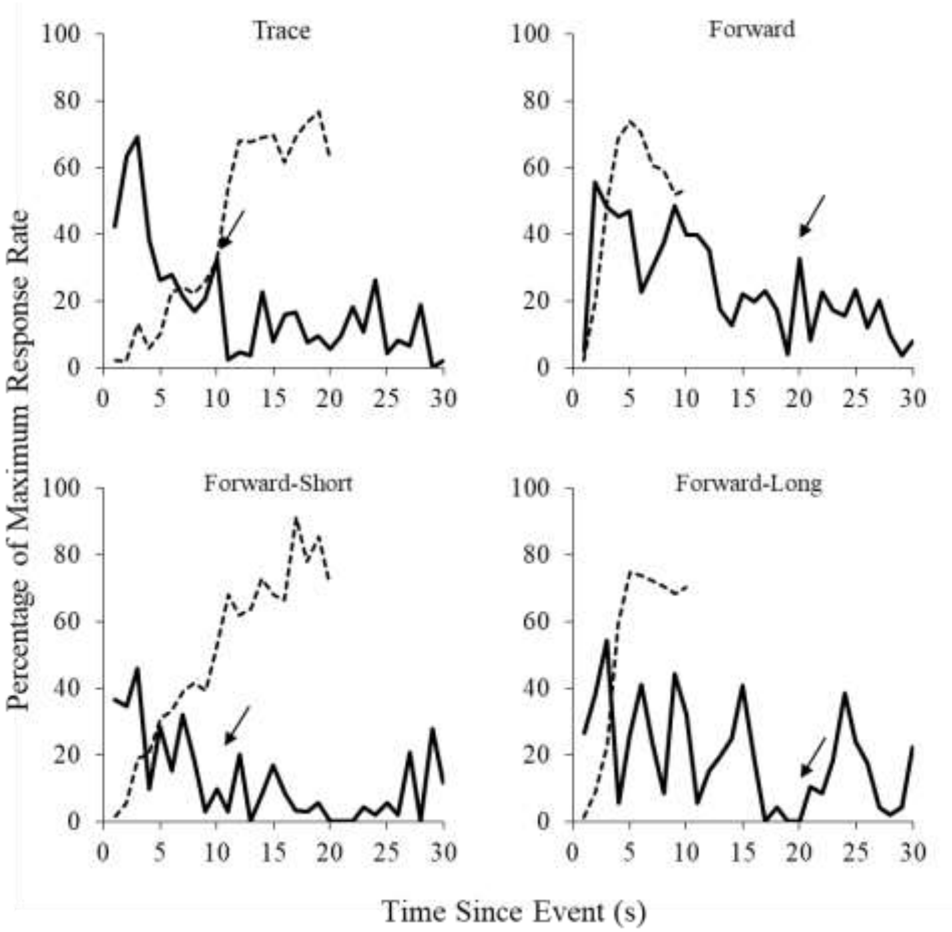

Figure 7. The percentage of the maximum response rate as a function of time since either CS1 onset on FOC trials (thin dashed lines) or CS2 onset on peak trials (heavy solid lines) for each of the four groups during second-order conditioning in Experiment 2. The expected time of US delivery relative to CS2 onset, assuming the formation of an integrated temporal map, is denoted by an arrow.

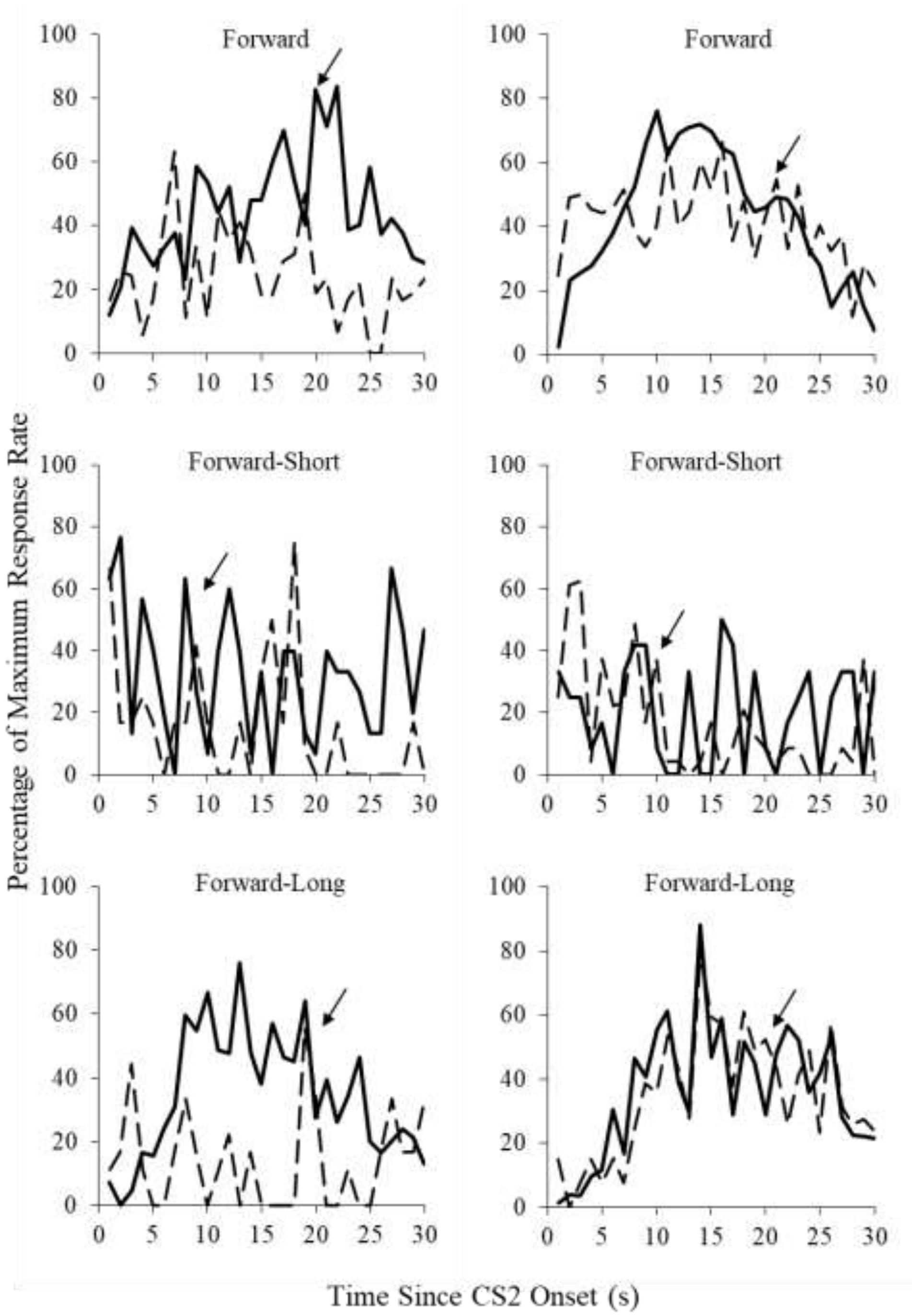

Figure 8. The percentage of the maximum response rate as a function of time since CS2 onset on peak trials for each of the groups Forward, Forward-Short, and Forward-Long during reinforced compound conditioning in Experiment 2. The left column displays these functions during the first ten sessions of reinforced compound conditioning, with either reinforced (heavy solid lines) or nonreinforced (thin dashed lines) SOC trials. The right column displays these functions during the last 15 sessions of the phase, where both reinforced SOC trials were delivered to all rats. The expected time of US delivery relative to CS2 onset, assuming the formation of an integrated temporal map, is denoted by an arrow.

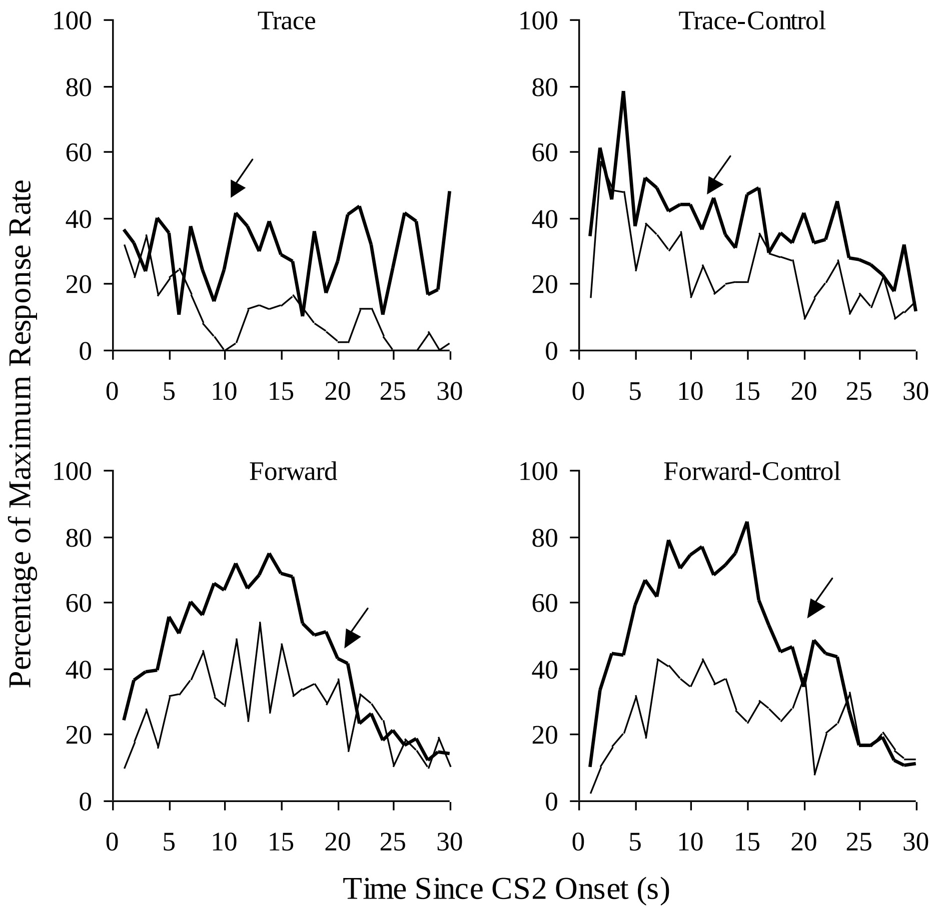

Figure 9. The percentage of the maximum response rate as a function of time since CS2 onset on peak trials for each of the four groups during Sessions 20-21 (thin solid lines) and Sessions 22-31 (heavy solid lines) of reinforced compound conditioning in Experiment 3. The expected time of US delivery relative to CS2 onset, assuming the formation of an integrated temporal map, is denoted by an arrow.

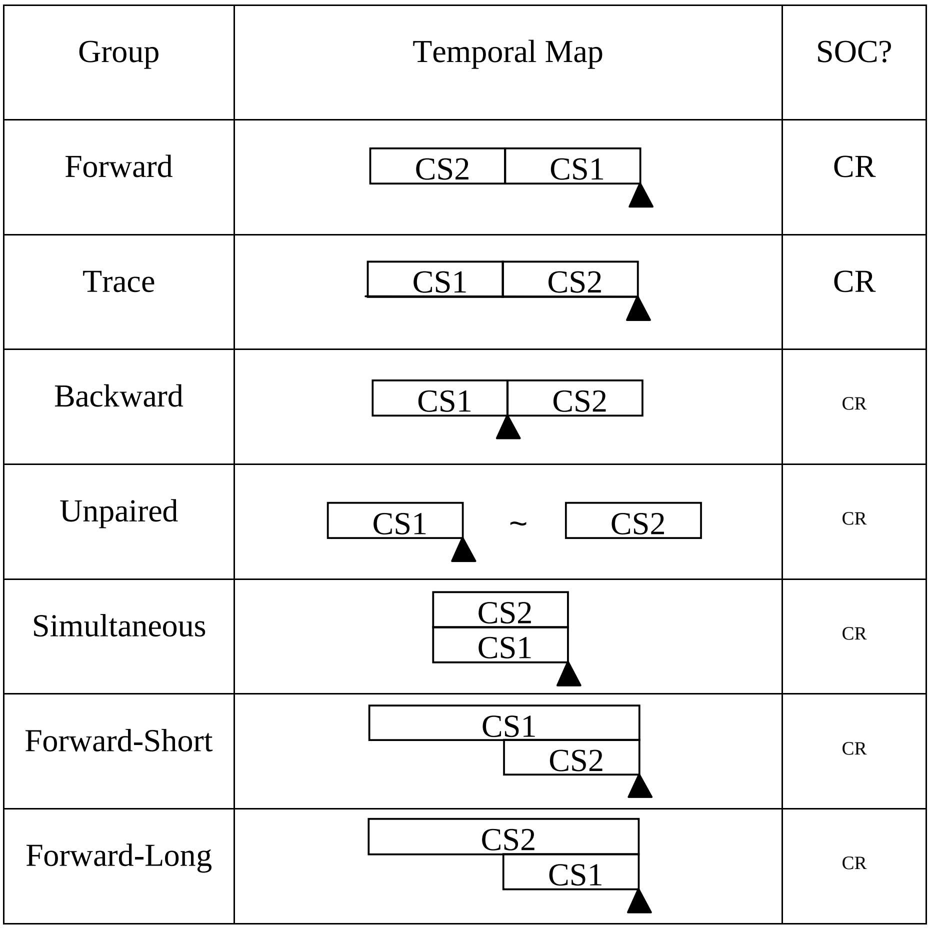

Figure 10. Temporal maps of procedures received by the different groups of rats in Experiments 1-3. The CSs are indicated by rectangles and the US by a triangle. The strength of SOC is denoted by font size (CR = good evidence of SOC; CR = weak evidence of SOC).

Footnotes

[1] Here and throughout a stimulus that appears in bold-italics denotes an expected event that does not occur. In the context of the temporal coding hypothesis, the ***US*** expectation develops through the process of temporal integration.

[2] The analysis was restricted to the initial 10 s because this was a usual duration of CS2 and thus provided the best index of second-order conditioning. Typical investigations of second-order conditioning would only test CS2 for its normal duration, but here the CS2 peak trials lasted for three times the normal duration. In subsequent experiments, the analysis window was always equal to the normal duration of CS2.